HEAVY METAL-INDUCED PROTEINS IN *CHLAMYDOMONAS REINHARDTII* AND *THALASSIOSIRA WEISSFLOGII* CELLS

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ABSTRACT

The photosynthetic algae *Chlamydomonas reinhardtii* and *Thalassiosira weissflogii* were exposed to several sublethal concentrations of Cd, Ni, and Pb. The cells were harvested at the end of the logarithmic phase of their growth curve. Heavy metal accumulation in the cells was determined in digested samples by atomic absorption spectrometry. After cell lysis, subcellular fractionation carried out by differential centrifugation and the isolated fractions were examined for their protein content, in order to identify and characterize proteins induced by heavy metal exposure. Size exclusion and ion exchange liquid chromatography were combined with polyacrylamide gel electrophoresis for protein identification. In this study, results are presented showing the induction of proteins due to the presence of heavy metals in the growing media.

Keywords: heavy metal-induced proteins, photosynthetic algae, phytochelatins, bioremediation

1. INTRODUCTION

Photosynthetic algae are in the basis of the nutrition chain, with very serious impact on the sustainability and health of water ecosystems. Pollution of water systems with heavy metals cause both adsorption on cell walls and insertion of these pollutants into the cells, with all the consequences to the higher forms of life, and finally to humans. On the other hand, the examination of the accumulation of heavy metals on algae living in a polluted environment may provide valuable information on the condition of the ecosystem, thus the exploration of the potential of algae to be used as bioindicators for heavy metal pollution is important [1,2]. In this study we examine the behavior of two photosynthetic algae, *Chlamydomonas Reinhardtii* (freshwater) and *Thalassiosira weissflogii* (seawater) exposed to a range of lead (0 – 25 mg/L), nickel (0 – 8.07 mg/L0 and cadmium (0 – 14.6 mg/L) concentrations. Growth curves of these two organisms were constructed for each heavy metal concentration, showing the differences on the tolerance of the two species to the heavy metals under examination. The percentage of the adsorbed vs. the total accumulated metal was estimated and the biochemical impact of the inserted metals in the cells was examined. For this, cells of the two species exposed to Pb, Ni or Cd were lysed and heavy metal-induced proteins were searched in the cytoplasm by SDS polyacrylamide gel electrophoresis [3]. Coomasie was used for the visualization of the proteins in the gels in order to examine the full protein profile, and sulfidryl group-containing proteins (mostly metallothioneins and phytochelatins), were quantified by Ellman method [4].

2. MATERIALS AND METHODS

Chlamydomonas reinhardtii cells were cultivated under continuous illumination at 25º C in TAP media supplied by acetic acid as organic carbon source, whereas *Thalassiosira weissflogii* cells

were cultivated under 14:10 light:dark and at 16º C in seawater with Guillard's (F/2) Marine Water Enrichment Solution (Sigma G0154). In both growing media, the appropriate amount of stock solution of nitrate salts of Pb, Ni, and Cd was added in order for the decided heavy metal concentration to be reached. Experiments for the construction of growth curves (optical density at 750 nm), were carried out in triplicates in individual 10-mL tubes, whereas cell biomass was acquired by using 1.5 L cultures.

The heavy metal content of the cells was determined by atomic absorption spectrometry: for total metal content, cells were harvested at the end of their logarithmic growth phase, and dried at 50º C until steady weight. Then, they were acid-digested and the digest was used for Pb, Ni, and Cd estimation. For in-cell metal content, before drying the cells were incubated for 10 minutes in the presence of 1 mM EDTA, and washed twice by centrifugation.

For cell lysis and fractionation, ultrasonication and differential centrifugation / ultracentrifugation were used. All experimental steps were carried out at 4º C. Lysates, Cytosol, Organelles, Heat Stable Proteins (HSP), and Heat Denaturable Proteins (HDP) were obtained with this method. All fractions were analysed for their protein profile by SDS-PAGE electrophoresis [3].

The sulfhydryl content of the cells was estimated by Ellman method [4].

3. RESULTS AND DISCUSSION

The growth curves of C. reinhardtii and T. weissflogii are presented in Figure 1. Higher concentrations of the three metals than those presented were lethal for the cells. From these curves it is obvious that *C. reinhardtii* is a much more tolerant organism in heavy metal pollution (at least for the studied metals, Pb, Ni and Cd) compared to *T. weisflogii*.

The relative amounts of Pb, Ni and Cd adsorbed on the cell walls and inserted in the cells of both organisms under study were determined by Atomic Absorption spectrometry: intact cells were incubated in a buffer with (+) or without (-) 1 mM EDTA. Then, the buffer was isolated from the cells by centrifugation (S+ and S-) and the cells were acid-digested (D+ and D-). The results of these analyses are presented in Figure 2.

These results show that compared to *C. reinhardtii, T. weissflogii* is a much better accumulator of all three heavy metals under examination. Also, heavy metal contents in the washing buffer and the cells, in the presence and absence of a strong chelator, indicate that, in all cases, most of the metal inserted the cells. These two results combined, could rationalize the observation that T. weissflogii was much more vulnerable to heavy metals: the higher amounts of the metals in the cells resulted in increased toxicity and cell death

The protein profiles of the *C. reinhardtii* cell lysates and the cell fractions (cytosols, organelles, HSP, HDP), were investigated by SDS-PAGE electrophoresis (Figure 3 A and B). In both gels, differences in the protein profiles between lysates and fractions of control cells and cells grown in the presence of Cd and Pb were observed. Indicatively, protein bands present in control lysates and absent (or not detected) in the lysates of the Cd and Pb-grown cells are indicated by top arrow, whereas more intensive protein bands in Cd and Pb- lysates are indicated by bottom arrow (Figure 3A). Similar observations can be made in the cell fractions presented in Figure 3B. Similar results were obtained from SDS-PAGE electrophoresis of cells grown in the presence of Ni (data not shown).

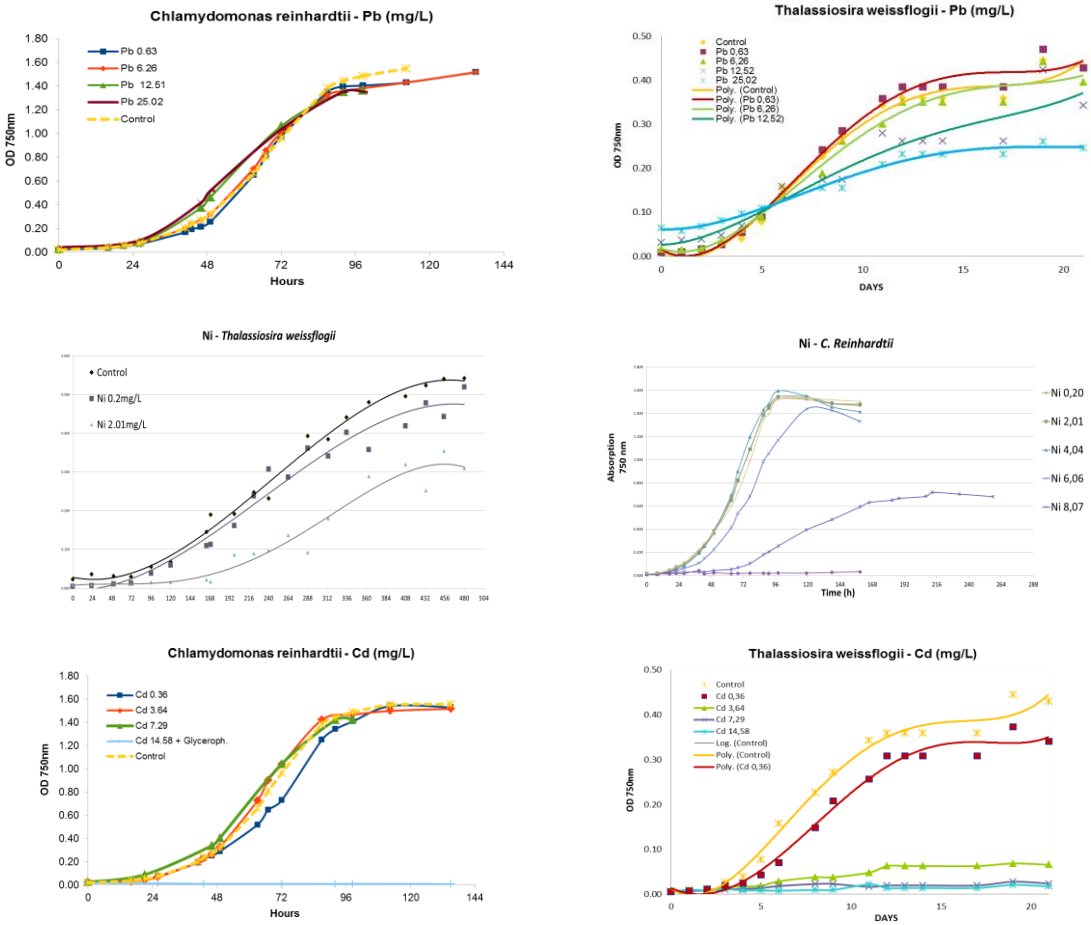


Figure 1. Growth curves for *C. reinhardtii* (left column) and *T. weissflogii* (right column), grown in the presence of various concentrations of Pb, Ni, and Cd (top to bottom, respectively).

The synthesis of SH- containing proteins (most likely phytochelatins?) in cells grown in the presence of Cd and Pb was investigated by Ellman method (Figure 4). In all cases, a significant increase of SH- containing proteins was observed in the presence of heavy metals. This result may indicate that the defensive system of *T. weissflogii* (at least in terms of defensive proteins synthesis) [5] is much less effective compared to *C. reinhardtii*, in agreement with the increased heavy metal-toxicity effects observed for it.

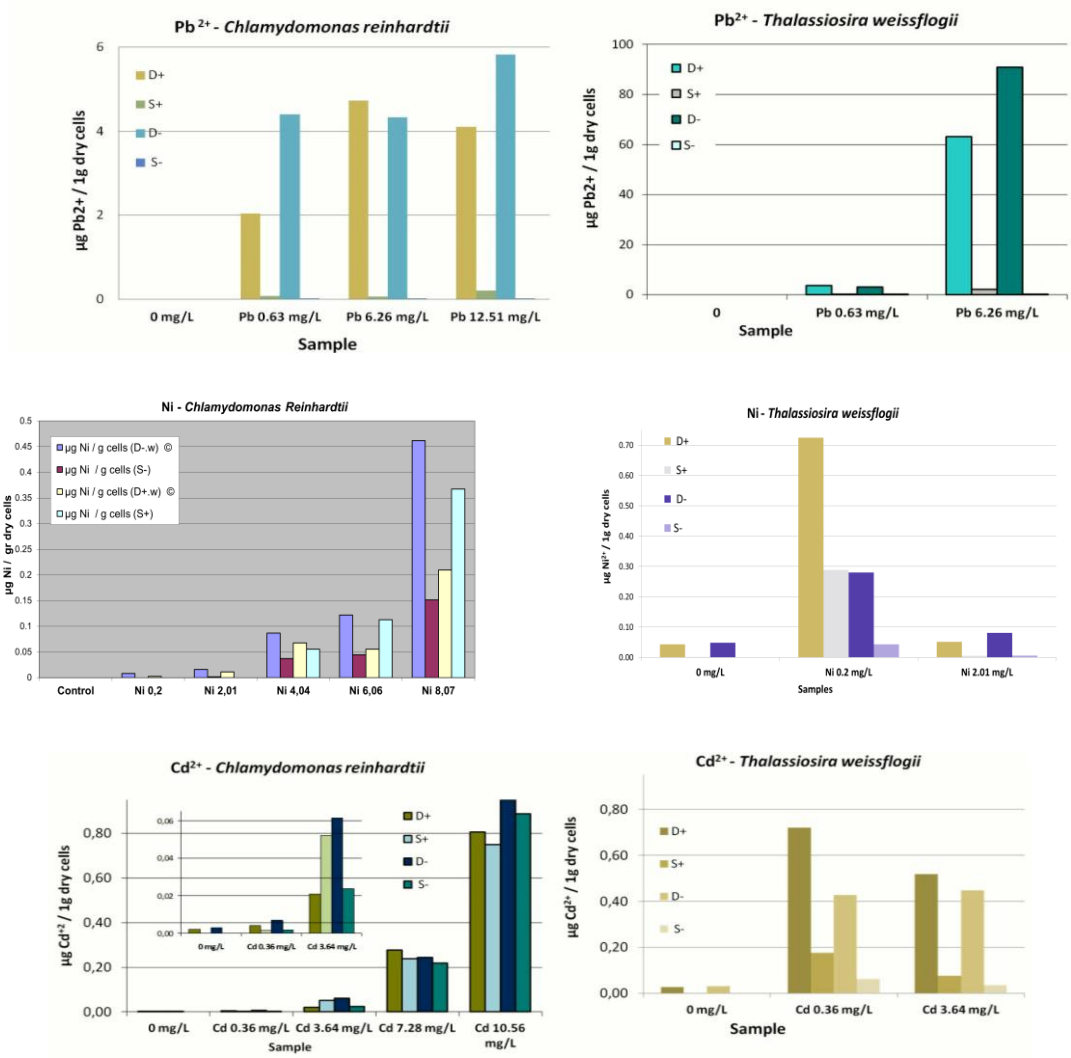
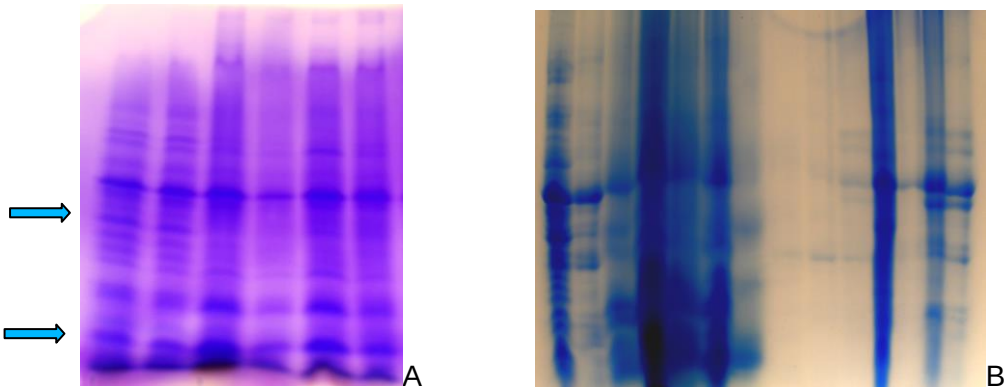


Figure 2. Metal content in washing buffer in the presence (S+) or in the absence (S-) of EDTA, and in the cells in the presence (D+) or in the absence (D-) of EDTA. The D- represents the total heavy metal content (adsorbed and inserted), D+ represents the inserted metal content and S+ represents the cell walls-adsorbed metals.



A B

Figure 3. Protein profiles in *C. reinhardtii* lysates (A) and cell fractions (B). A. Lanes 1,2 control, lanes 3,4 +Cd, lanes 5,6 +Pb. B. Lane 1 control lysate, lanes 2,3,14 cytosols control, +Pb, +Cd, respectively, lanes 4,5,6 organelles control, +Pb, +Cd, lanes 8,9,10 HSP control, +Pb, +Cd, lanes 11,12,13 HDP control, +Pb, +Cd.

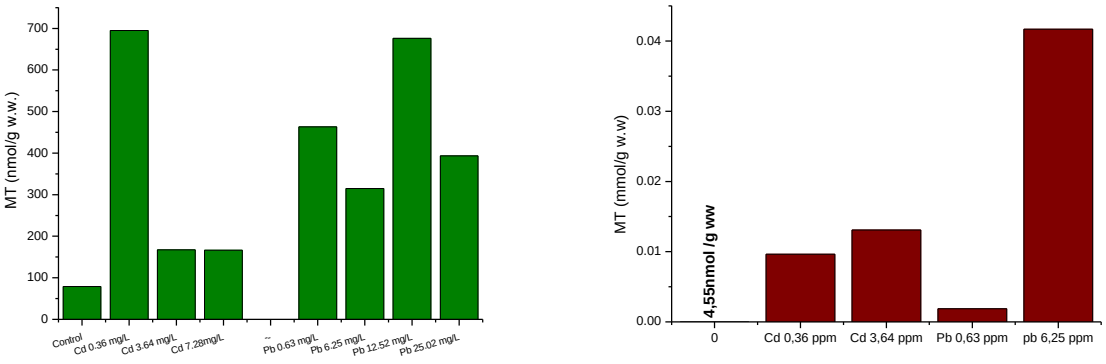


Figure 4. SH- containing proteins in control, +Cd and +Pb lysates of *C. reinhardtii* (left) and *T. weissflogii* (right).

REFERENCES

1. Assimakopoulos V., Moussiopoulos N. and Apsimon H.M. (2000), Effects of Street Canyon Geometry on the Dispersion Characteristics in Urban Areas, 16th IMACS World Congress 2000, August 21-25 2000 Lausanne, Switzerland Perales-Vela, H.V., Pena-Castro, J.M., Cansares-Villanueva, R.O. (2006), Heavy Metal Detoxification in Eukaryotic Microalgae, *Chemosphere*, **64**, 1-10.
2. Torres, M.A., Barros, M.P., Campos, S.C.G., Pinto, E., Rajamani, S., Sayre, R.T., Colepicolo, P., (2008), Biochemical Biomarkers in Algae and Marine Pollution: A Review, *Ecotoxicol. Environ. Safety,* **71**, 1-15.
3. Simpson, R. J., (2003), Proteins and Proteomics: A Laboratory Manual, Cord Spring Harbor Laboratory Press, NY.
4. Ellman, G. L., (1959),Tissue sulfhydryl groups, *Archives in Biochemistry Biophysiology*, **82,** 70-77.
5. Cobbett, C., Goldsbrough, P., (2002), Phytochelatins and Metallothioneins: Roles in heavy metal detoxification and homeostasis, *Ann. Rev. Plant Biol*., **53**, 159-182.

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